



**QUEEN'S  
UNIVERSITY  
BELFAST**

## **Fine mapping of genes determining extrafusal fiber properties in murine soleus muscle**

Carroll, A., Cheng, R., Collie-Duguid, E., Meharg, C., Scholz, M., Fiering, S., Fields, J., Palmer, A., & Lionikas, A. (2017). Fine mapping of genes determining extrafusal fiber properties in murine soleus muscle. *PHYSIOLOGICAL GENOMICS*, 49(3), 141-150. <https://doi.org/10.1152/physiolgenomics.00092.2016>

**Published in:**  
PHYSIOLOGICAL GENOMICS

**Document Version:**  
Peer reviewed version

**Queen's University Belfast - Research Portal:**  
[Link to publication record in Queen's University Belfast Research Portal](#)

**Publisher rights**  
© 2016 The American Physiological Society  
This work is made available online in accordance with the publisher's policies. Please refer to any applicable terms of use of the publisher.

**General rights**  
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

**Take down policy**  
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact [openaccess@qub.ac.uk](mailto:openaccess@qub.ac.uk).

1 **Fine mapping of genes determining extrafusal fiber properties in murine soleus**  
2 **muscle**

3 Carroll AM<sup>1,2</sup>, Cheng R<sup>3</sup>, Collie-Duguid ESR<sup>1,4</sup>, Meharg C<sup>5</sup>, Scholz ME<sup>1</sup>, Fiering S<sup>6</sup>, Fields  
4 JL<sup>6</sup>, Palmer AA<sup>7</sup>, Lionikas A<sup>1</sup>.

5

6 Affiliation:

7 <sup>1</sup>School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Foresterhill  
8 Aberdeen, AB25 2ZD, UK

9 <sup>2</sup>The New Zealand Institute for Plant & Food Research Limited, Batchelar Road, Private Bag  
10 11600, Palmerston North 4442, New Zealand

11 <sup>3</sup>Research School of Biology, The Australian National University, 134 Linnaeus Way, Acton,  
12 ACT 2601, Australia

13 <sup>4</sup>Centre for Genome Enabled Biology and Medicine, University of Aberdeen, Old Aberdeen,  
14 Aberdeen, AB24 3RY, UK

15 <sup>5</sup>Institute for Global Food Security, Queen's University Belfast, 18-30 Malone Road, Belfast,  
16 BT9 5BN, UK

17 <sup>6</sup>Department of Microbiology/immunology, Geisel School of Medicine at Dartmouth,  
18 Hanover, NH 03755, USA

19 <sup>7</sup>Department of Psychiatry, University of California San Diego, 9500 Gilman Drive, Mail Code  
20 0667, BRF2 3A24, La Jolla, CA 92093, USA

21

22

23

24

25

26

27 **Abstract**

28 **Introduction.** Muscle fiber cross-sectional area (CSA) and proportion of different fiber types  
29 are important determinants of muscle function and overall metabolism. Genetic variation  
30 plays a substantial role in phenotypic variation of these traits, however, the underlying genes  
31 remain poorly understood.

32 **Aims.** This study aimed to map quantitative trait loci (QTL) affecting differences in soleus  
33 muscle fiber traits between the LG/J and SM/J mouse strains.

34 **Methods.** Fiber number, CSA, and proportion of oxidative type I fibers were assessed in the  
35 soleus of 334 genotyped female and male mice of the F<sub>34</sub> generation of advanced intercross  
36 lines (AIL) derived from the LG/J and SM/J strains. To increase the QTL detection power,  
37 these data were combined with 94 soleus samples from the F<sub>2</sub> intercross of the same  
38 strains. Transcriptome of the soleus muscle of LG/J and SM/J females was analysed using  
39 microarray.

40 **Results.** Genome-wide association analysis mapped 4 QTL (genome-wide  $p < 0.05$ )  
41 affecting the properties of muscle fibers to Chromosome 2, 3, 4 and 11. A 1.5-LOD QTL  
42 support interval ranged between 2.36 Mb and 4.67 Mb. Based on the genomic sequence  
43 information, functional and transcriptome data, candidate genes were identified for each of  
44 these QTL.

45 **Conclusion.** Combination of analyses in F<sub>2</sub> and F<sub>34</sub> AIL populations with transcriptome and  
46 genomic sequence data in the parental strains is an effective strategy for refining QTL and  
47 nomination of the candidate genes.

48

49 Key words: skeletal muscle, muscle fiber types, genetic variation

50

## 51 **Introduction**

52 Skeletal muscle plays a broad range of biological functions including locomotion,  
53 thermoregulation, respiration, postural support, protection of bones and viscera; as well as  
54 serving as a source of amino acids in times of starvation or disease. Muscle tissue in  
55 livestock also provides an essential source of dietary proteins. In humans, there is more  
56 than a 2-fold difference in muscle mass between individuals of similar age and same sex (3,  
57 33). This is the outcome of variability in the number of muscle fibers and their size (51).  
58 These differences are of clinical relevance. Variability in muscle mass significantly impacts  
59 energy expenditure (58), influencing preponderance to obesity. In addition, individuals with  
60 lower muscle mass may be more vulnerable to impairment of these vital functions due to  
61 aging and/or disease related muscle loss. It has recently been reported that there is a  
62 positive association between muscle mass and longevity in older adults (66).

63  
64 Human skeletal muscles are mainly comprised of a mixture of type I, IIA and IIX muscle  
65 fibers (62). The number of fibers, their size and varying proportions of the fiber types affect  
66 morphological and functional properties of the muscle (6). A larger diameter of the fibers  
67 and higher number of fibers typically leads to augmented muscular strength and power (25,  
68 28). The proportion of type I muscle fibers is a factor determining success in endurance  
69 sporting events (15, 18) and overall metabolism in humans (24, 29, 44, 74). In livestock,  
70 proportion of oxidative type I fibers is associated with meat quality (65).

71  
72 In humans, genetic factors account for around half of the variation in strength (19, 24, 74)  
73 and the upper limit heritability is even greater (over 0.9) for muscle mass (26). Heritability  
74 estimates of proportion of type I fibers is also high, ranging between 0.4 and 0.9, indicating  
75 that genetic factors play an important role in determining muscle fiber properties (37, 63).  
76 Effects of genetic factors on muscle fibers have also been demonstrated in mouse (20, 22,

59), cattle (68), sheep (10, 38) and pig (71). However, the specific genes underlying these effects remain largely elusive.

79

Attempts at mapping the polygenic architecture of muscle fiber properties in mouse (11), pig (17, 43, 52, 55, 77), cattle (1) and carp (80) have been made. A number of QTL have been identified in these studies. However, the resolution achieved in the  $F_2$  population is not adequate for reliable nomination of the candidate genes in the majority of the QTLs of polygenic traits. The mouse soleus muscle (primarily consists of type I and IIA fiber types), closely resembles the fiber type composition of human skeletal muscles (primarily comprised of type I, IIA and IIX fiber types), and is therefore a particularly interesting experimental model. In our previous study, we mapped soleus muscle fiber traits in an  $F_2$  intercross between the LG/J and SM/J laboratory mouse strains (11). These strains differ in a number of muscular phenotypes, with the LG/J strain displaying a greater proportion of type I fibers, and a greater cross-sectional area (CSA) of type I and IIA muscle fibers. We identified in that study three significant QTLs contributing to the difference in the CSA of muscle fibers between LG/J and SM/J strains (11). Regions of conserved synteny from the identified loci were also implicated in fiber phenotypes in pig supporting the importance of these genomic regions in determining muscle fiber properties. However, the exact genes underlying their effects remain to be determined.

96

Integration of advanced study populations, high throughput gene expression technology and increasing availability of knockout models aid identification of the causative genes.

Nomination of the genes underlying QTL effects can be facilitated by improving the mapping resolution, and by utilising genomic sequence and transcriptome information. Advanced intercross lines (AIL) have been proposed as a powerful population for mapping QTLs (16). It has been demonstrated recently that a joint  $F_2$  and AIL analysis can combine the

103 advantages of both mapping populations by increasing the power to detect QTLs and  
104 achieving a higher mapping resolution of various traits in mice (13, 47). Additionally, testing  
105 for differences in specific gene expression has led to several nominations of quantitative trait  
106 genes (30, 35). For validation of such candidate genes, phenotypic effects of relevant  
107 alleles can be examined in experimental populations where these alleles segregate albeit on  
108 a different genetic background. In addition, available knockout models offers particularly  
109 attractive option for validation experiments.

110 In the present study we aimed to fine-map QTL and nominate candidate genes affecting the  
111 CSA and proportion of oxidative type I fibers in the soleus muscle in a combined analysis of  
112  $F_2$  and  $F_{34}$  AIL mice, and by cross referencing QTL data with soleus transcriptome profiles in  
113 the parental strains. Further filtering of the emerged candidates was carried out in an  
114 independent AIL and a knockout model.

115

## **Methods**

### **Muscle Samples**

This study was carried out on soleus muscles dissected from females and males of the F<sub>34</sub> advanced intercross lines (AIL) of the LG/J and SM/J inbred strains. Animals were maintained as previously described (13) and sacrificed at 94 ± 4 days. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Chicago. Soleus muscle samples from F<sub>34</sub> AIL mice described in our previous study (47) were subjected to histological analyses. The final sample size used in the present study was 334 F<sub>34</sub> mice, 142 females and 192 males, after discarding samples of poor tissue quality. A set of 94 F<sub>2</sub> samples (38 females and 56 males) described in our previous study (11) was also used in order to increase the QTL detection power.

In addition, we also analysed soleus muscle samples for two hypothesis driven studies aimed at testing the effects of identified candidate genes on percentage of oxidative, type I fibers. First, we examined solei samples from the *Chd6* ATPase knockout (n=6), heterozygous (n=4) and wild type (n=4) females. The generation of the *Chd6* mutant mice has been previously reported (40). Briefly, the genetic manipulation generated an allele with the ATPase domain of *Chd6* (exon 12) flanked by loxP sites so that the action of Cre recombinase would delete this domain. The mice were mated to a germline Cre-expressing strain (Jackson lab strain 003465) to delete both exon 12 and the neomycin resistance marker used for the targeting. Subsequently breeding generated the *Chd6* ATPase knockout mice utilized in the present study. Second, solei of the advanced intercross mice (generations F<sub>9</sub>-F<sub>12</sub>), all homozygous carries of the C57BL/6J (n=22) or DBA/2J (n=23) alleles at the region harbouring the *Alad* gene were selected from the tissue bank of our previous study (9).

### **Phenotype assessment**

The soleus muscles were frozen in isopentane cooled in liquid nitrogen. Transverse sections from the belly of the muscle were cut at a thickness of 10  $\mu$ m with a cryotome (Leica CM1850UV) at -20°C. The muscle sections were subjected to ATPase staining (acid pre-incubation, pH 4.47) to distinguish between fiber types (8). Microscopic images of stained sections were taken at x5 and x20 magnification.

The following phenotypes were assessed: muscle fiber number (type I and IIA) and percent of type I muscle fibers, cross-sectional area (CSA) of type I and type IIA fibers (**Figure 1**). Muscle fiber traits were manually analysed using ImageJ software (NIH-version 1.43). 25 measurements of each fiber type were taken using the freehand selection tool at x20 magnification to obtain a value representing the mean CSA of type I or type IIA fibers for that muscle. This was deemed as a representative sample by empirical testing as described previously (11). Total number of type I and type IIA muscle fibers were counted using the ImageJ cell counter plugin on x5 magnification images. As all fibers in mouse soleus pass through the belly of the muscle (69), this method provides an accurate estimate of the number of fibers constituting the muscle. Total number of type I fibers and total number of type IIA fibers were counted, permitting derivation of percentage of type I fibers. Over the course of the study ~200,000 muscle fibers were counted and ~6,700 fibers measured for CSA.

#### Statistical analyses

The GraphPad Prism version 5.0 statistical package was used (GraphPad software, La Jolla, CA). Data are presented as mean  $\pm$  SD, unless otherwise stated. The CSA of type I and type IIA fibers were analysed using a two-way (sex and fiber type) paired-measures (type I and type IIA fibers) ANOVA.

#### Genotyping and QTL mapping

Mice were genotyped using a custom designed SNP array that included 4,610 polymorphic SNPs that were approximately evenly distributed across the genome, as described



previously (13). The genome-wide association analysis was performed in the combined population of the  $F_{34}$  and recently published  $F_2$  intercrosses (11) using the R package QTLRel (12). This software accounted for the complex relationships (e.g., sibling, half-sibling, cousins) among the  $F_{34}$  mice by using a mixed model, as previously described (12, 13). Due to the sex differences in muscle mass in these mice (47), and the discovery of sex specific QTL in other studies (45, 46), we included sex as an additive and interacting covariate. Threshold of significance was estimated by 1000 permutations (14). We defined the support interval for each QTL as the 1.5-LOD drop off on either side of the peak marker. This interval was expressed in physical map position (Mb) by using the nearest genotyped SNP that flanked the support interval, based on the mouse genome build GRCm38.p3.

#### Transcriptome analysis

Soleus muscle tissues from 92-day old LG/J and SM/J females (n=3 of each strain) were used. RNA was isolated using TRIzol (Invitrogen Life Technologies, Carlsbad, CA) followed by purification and DNase digestion using RNeasy minikits (Qiagen, Venlo, Netherlands) according to the manufacturer's instructions. Quantification of total RNA was performed on a NanoDrop spectrophotometer (Thermo Scientific) and quality tested on an Agilent Tapestation with R6K Screentapes (RIN  $\geq 7.3$ ). Generation of sense strand cDNA from purified total RNA (Ambion<sup>®</sup> WT expression kit, Ambion, Austin, Texas) followed by fragmentation and labelling (GeneChip WT labelling kit, Affymetrix, Santa Clara, CA) were performed according to the manufacturer's instructions. Hybridisation, washing, staining and scanning of microarrays were carried out on Affymetrix Mouse Gene 2.0 ST microarrays according to the manufacturer's standard protocols using a GeneChip Fluidics station 450 and GCS3000 scanner (Affymetrix<sup>®</sup>, Santa Clara, CA). Microarray data are available in the ArrayExpress database ([www.ebi.ac.uk/arrayexpress](http://www.ebi.ac.uk/arrayexpress)) under accession number E-MTAB-5290.

Data pre-processing and quality control analysis was performed using Affymetrix® Genechip® Expression Console v1.2. Probe cell intensity data on the Mouse Gene 2.0 ST array (CEL files) were processed using the RMA16 algorithm (Affymetrix, Santa Clara, CA, USA) which fits a robust linear model at probe level by employing background correction, quantile normalisation of log2 transformed data and summarisation to probe level data (CHP files, 41,345 probe sets).

Data was analysed for differentially expressed genes in Partek® Genomics Suite® version 6.6, build 6.15.0730 (Partek Inc., St Louis, MO) using a *Mus musculus* build mm10 annotation file for Mouse Gene 2.0 ST microarrays (MoGene-2\_0-st-v1.na35.mm10). CEL files (Expression Console v 1.2, Affymetrix, Santa Clara, CA) were imported to Partek Genomics Suite v 6.6 and processed using RMA normalisation with background correction of log2 transformed data and probe set summarisation by median polish. Differential expression analysis between the LG/J and SM/J strains of all genes (n=41,345 transcript clusters) was determined by 1-way ANOVA with Storey's FDR, and q-value  $\leq 0.05$  considered significant (n=819 genes differentially expressed  $\geq 1.2$  fold; see **Supplementary Table 1**).

To assess transcription of positional candidate genes in each strain, a hypothesis driven analysis of differential gene expression was performed between the LG/J and SM/J strains on all genes mapping to the support interval defined for each QTL in the GWAS described above. Using Partek Genomics Suite v.6.6, a total of 159 genes that were represented on the mouse Gene 2.0ST microarray, were identified in *Mus musculus* genome build GRCm38, mm10 within mapping co-ordinates Chr2:158908559–162608559 (26 genes), Chr3:33308451–35708451 (15 genes), Chr4:57605946–62913639 (77 genes) or Chr 11:279000000–315000000 (41 genes). 1-way ANOVA identified differentially expressed genes between the LG/J and SM/J strains ( $P < 0.05$ ). Fold change was calculated using the geometric mean of samples in each group.

## 220 Candidate genes

221 Nomination of the candidate genes was based on the following three criteria. First, we  
222 scrutinized polymorphisms in positional candidates between the LG/J and SM/J strains. The  
223 emphasis was on the indels and SNPs that would affect the coding sequence and lead to  
224 changes in amino acids. To assess whether amino acid substitution would influence the  
225 function of a protein, evolutionary conservation at the site of substitution and properties of  
226 substituted amino acids were considered using three different bioinformatics tools as  
227 described by Nikolskiy and colleagues (56). Second, we examined expression of positional  
228 candidates across a panel of over ninety mouse tissues and cell types available in BioGPS  
229 GeneAtlas MOE430, gcrma dataset (79). This analysis permits a quantitative comparison of  
230 transcript abundance of a gene between tissues. We considered that an abundant  
231 expression in skeletal muscle lineage, i.e. muscle tissue and/or C2C12 myogenic cell line,  
232 implies functional and/or structural relevance of a gene in this tissue. Third, we compared  
233 gene expression levels in the soleus muscle between the two strains as described in the  
234 previous section. Expression difference in this analysis might point at the strain-specific,  
235 genotype-dependent mechanism underlying the phenotypic difference.

236

## 237 Results

### 238 Phenotypic analyses

239 **CSA.** Cross section analysis of soleus muscle fibers were done on mice of both sexes from  
240 the F<sub>34</sub> cohort. For muscle fiber cross-sectional area, we observed a statistically significant  
241 sex by fiber type interaction (P<0.0001). In the female F<sub>34</sub> mice there was no significant  
242 difference between type I and type IIA muscle fiber areas (913 ± 229 μm<sup>2</sup>, n=140; and 952 ±  
243 242 μm<sup>2</sup>, n=140 respectively; P=0.2). However, there was a significant difference within the  
244 males, with the type I muscle fiber area being smaller than IIA fiber area (1084 ± 238 μm<sup>2</sup>,

n=187; and  $1215 \pm 294 \mu\text{m}^2$ , n=187 respectively;  $P < 0.0001$ ). Muscle fiber area was lower in females than males for type I CSA, ( $P < 0.0001$ ) and type IIA CSA ( $P < 0.0001$ ).

**Percentage of type I fibers.** The number of type I fibers as a percentage of total fibers varied substantially between individuals, ranging from 30% to 67% in females, and from 26% to 59% in males (**Figure 1**) and was greater in females than males ( $46 \pm 8\%$ , n=142; and  $39 \pm 6\%$ , n=189; respectively;  $P < 0.0001$ ).

**Total fiber number.** No difference was observed in the total soleus fiber number between females and males ( $646 \pm 102$ , n=120, and  $667 \pm 105$ , n=177, respectively;  $P = 0.0979$ ).

#### QTL analyses

Muscle fiber traits approximated the normal distribution in both the  $F_2$  and  $F_{34}$  population (**Supplementary Figure 1**). We identified significant QTL (at the 1% or 5% level of genome-wide statistical significance) (39) for CSA of type I and type IIA fibers and the percentage of type I fibers. We also identified chromosome-wide significant QTL for CSA of type I and type IIA fibers, the percentage of type I fibers and total fiber number (**Table 1**). The size of the support interval of these QTL ranged from 0.4-40.7 Mb, with a median of 4.6 Mb.

The QTL at the genome-wide level of significance for CSA of type I and type IIA fibers on chromosome 3 was named *Mfq5*. The QTL at the genome-wide level of significance for the percentage of type I fibers on chromosome 2 and 4 were named *Mfq4* and *Mfq6*, respectively. The SM/J allele conferred a greater percentage of type I fibers at *Mfq4*, and a greater CSA at *Mfq5*. The LG/J allele conferred a greater percentage of type I fibers at *Mfq6* locus.

A significant QTL affecting CSA of type I and type IIA fibers was also detected on chromosome 11 (**Figure 2**) within the same region as locus *Mfq3*, previously identified in the  $F_2$  intercross of the same parental strains (11). The QTL exhibited male-specificity in both

type I and IIA fibers of the  $F_{34}$  mice (**Figure 3**). Because this QTL recapitulated properties of the *Mfq3* locus, which we also found to be male specific in the  $F_2$  population, we concluded that the same locus has been refined in  $F_{34}$  and did not assign a new name for this QTL. Earlier reported *Mfq2* locus has been refined in a similar manner; a QTL on chromosome 6 affecting CSA of type I and type IIA fibers (at 1% chromosome specific threshold) was engulfed by the support interval of *Mfq2* and also replicated its increasing allele, LG/J, in both females and males (not shown).

#### Gene expression analyses

We hypothesized that each identified QTL harbours one or more genetic variants that drive phenotypic differences by means of differential gene expression. Hypothesis driven analysis of differential expression in soleus muscle was performed between LG/J and SM/J strains for the genes in the most robust QTLs affecting fiber CSA or % Type I fibers (*Mfq3*, *Mfq4*, *Mfq5* and *Mfq6*). The Mouse Gene 2.0 ST expression array contains 159 genes residing within the support intervals of these QTLs (**Supplementary Table 2**). Twenty genes (**Table 2**) showed evidence of differential expression (ANOVA,  $p \leq 0.05$ ), 2 of which, *Alad* and *Hdhd3*, were significant after correction for the multiple testing problem (Storey's FDR  $q \leq 0.05$ ). Compared to other tissues and cell types, expression of differentially expressed genes *Mafb*, *Acyp2* and *Mtif2* (**Table 2**), is particularly enriched in skeletal muscle (BioGPS, Mouse MOE430 gene expression data).

#### Genomic analyses

Positional candidates with non-synonymous polymorphisms provide a plausible genetic cause for the phenotypic differences. Based on the genomic sequence of the LG/J and SM/J strains (56), we identified 21 genes in the QTL regions with non-synonymous polymorphisms predicted to affect protein function by at least one out of three algorithms used in the analysis (**Supplementary Table 3**). Four of those genes (*Mfq3*: *Mtif2*, *Rtn4*, *Psme4*; *Mfq5*: *Dnajc19*) are prioritized further because of their preferential expression in

muscle lineage (differentiated muscle and/or C2C12 myoblasts) compared to other tissues and cell types. Among those, the *Mtlf2* gene differs by 3 (rs26871496, rs26871494, rs29436813) and *Rtn4* by 9 (rs29473364, rs29469198, rs13463765, rs29465940, rs26857726, rs26857725, rs29474377, rs26857722, rs26857721) amino acids between the two strains. At all SNPs the SM/J strains carries reference while the LG/J strain the alternative allele.

#### Candidate gene analyses

The *Chd6* gene emerged as a differentially expressed positional candidate for the *Mfq4* locus affecting percentage of type I fibers (**Table 2**). To test its effect we examined soleus muscles of *Chd6* knockout, heterozygous and wild type littermates. This analysis however revealed that the genotype of the animals did not have a significant effect ( $P=0.30$ ) on the percentage of type I fibers (**Figure 4**).

The *Alad* gene emerged as a candidate for another locus affecting proportion of type I fibers, *Mfq6*. In the animals of an advanced intercross between the C57BL/6J and DBA/2J strains (these strains carry one or three copies of *Alad*, respectively (3)), we examined if percentage of type I fibers was genotype-dependent. The analysis revealed no difference in the percentage of type I fibres between the carriers of the C57BL/6J and the DBA/2J alleles,  $42 \pm 7\%$  and  $42 \pm 8\%$ , respectively.

## 314 **Discussion**

315 A previous study on muscle weight in LG/J and SM/J strains identified a two-fold difference  
316 in soleus muscle size (47). We then explored the cellular and genetic mechanisms  
317 contributing to this phenomenon, finding that the difference was largely due to the CSA of  
318 muscle fibers and we mapped QTL affecting muscle fiber traits in an F<sub>2</sub> intercross between  
319 the LG/J and SM/J strains (11). The present study, which utilizes the F<sub>34</sub> advanced  
320 intercross, verified, refined and expanded our earlier findings.

321 A number of studies have previously reported the effects of *Stat5a* and *Stat5b* (36), *Pgc-1α*  
322 (42), *Ky* (4), myostatin (54), leptin (61), calcineurin (76), *Sod1* (5), alpha-actinin-3 (50),  
323 dystrophin (7), *Tbx15* (41) and IIB myosin heavy chains (2) genes on muscle fiber area in  
324 knockout or mutant models. In addition, *Pgc-1α* (75), calcineurin (76), *Foxo1* (34) and  
325 myostatin (20) are reported to affect the proportion of muscle fiber types. However, the  
326 genomic positions of these genes have not been linked to muscle fiber differences between  
327 the LG/J and SM/J strains, implicating involvement of novel genes.

328 **Muscle fiber number.** The number of fibers is an important determinant of muscle size and  
329 functional properties. It is set during embryogenesis and the first post-natal week in mice  
330 (78). The number of muscle fibers in males (667 ± 105) and females (646 ± 102) of the F<sub>34</sub>  
331 population was comparable to that observed in the soleus of the F<sub>2</sub> population (645 ± 102  
332 and 595 ± 107, respectively), and within the range of the fiber count observed in solei of a  
333 variety of different strains of mice ~250~900 fibers (32, 49, 57, 70, 72).

334 From these data it emerged that males and females are born with a similar number of fibers  
335 in soleus muscle, and that the sex difference in muscle weight (males have approximately  
336 30% larger soleus than females) is due to the difference in fiber size. Comparison of the  
337 parental strains also revealed a similar number of fibers (11), despite the 2-fold difference in  
338 soleus weight (47), demonstrating that size rather than number of fibers determines variation  
339 in muscle weight between the LG/J and SM/J strains.

**Fiber area.** The CSA of muscle fibers in the LG/J strain is 49% to 90% greater than the corresponding fibers in the SM/J strain, indicating that this variable accounts for a large portion of the muscle mass difference between the strains (47).

The area of type I ( $1084 \pm 238 \mu\text{m}^2$  and  $913 \pm 229 \mu\text{m}^2$  for males and females, respectively) and type IIA ( $1215 \pm 294 \mu\text{m}^2$  and  $952 \pm 242 \mu\text{m}^2$ , respectively) of the  $F_{34}$  mice was comparable to the corresponding fiber area of the  $F_2$  mice of the same lineage (11) and it is within the range reported for the type I, between  $920 \mu\text{m}^2$  and  $1780 \mu\text{m}^2$  (32, 57, 70), and type IIA fiber area, between  $700 \mu\text{m}^2$  and  $1400 \mu\text{m}^2$  (32, 70), in various inbred mouse strains.

**Percentage of type I fibers.** The percentage of type I fibers in male ( $39 \pm 6\%$ ) and female ( $47 \pm 8\%$ )  $F_{34}$  mice were also within the range of previous studies, which showed the percentage of type I fibers in the soleus muscle fluctuates between ~25 and ~66% (32, 57, 70).

In the  $F_{34}$  mice we replicated our observation in the  $F_2$  population that the percentage of type I fibers was significantly greater in females than males. This sex difference is also observed in various human muscles where, in general, women have a higher percentage of type I muscle fibers than males (27, 53, 60, 64, 67). The phenomenon is likely to be explained, at least partly, by the effect of androgens; castration leads to a higher percentage of type I fibers in the soleus of male mice (73).

**Validation and refinement of genetic architecture.** In the present study, we validated and refined the genetic architecture of muscle fibers identified in an  $F_2$  intercross between the same parental strains (11). In order to increase QTL detection power, we increased sample size by combining the  $F_{34}$  and  $F_2$  data. The median mapping resolution of 4.6 Mb for muscle fiber QTLs was comparable with 3.7 Mb of muscle weight QTLs obtained in the same population albeit using ~1,600 fewer genetic markers than in the present analysis (47). A genome-wide significant QTL identified in the present study between 27.9 Mb and 31.4 Mb



on chromosome 11 (**Table 1**) overlapped with a significant QTL, *Mfq3*, mapped in the  $F_2$  population (11). In addition to the chromosomal location, the increasing allele of this locus (LG/J) and its male-specific effect (**Figure 3**) were also replicated in  $F_{34}$ , suggesting that the same gene(s) were involved in two different populations and permitting us to refine the *Mfq3* locus from 51.6 Mb to 3.57 Mb. The presence of two satellite QTL proximal of the refined *Mfq3* (**Table 1**) suggests that the QTL observed in the  $F_2$  population (11) might have been an outcome of up to three linked loci.

The recently reported “mini-muscle” locus, mapped to 67.1–70.2 Mb on chromosome 11, affects muscle fiber area and proportion of fiber types (21-23). However, the mutation responsible for the “mini-muscle” phenotype maps to an intron of *Myh4* gene located at 67.2 Mb (31), between the support intervals of two adjacent QTLs affecting fiber type between the LG/J and SM/J strains (**Table 1**). Together, these data suggest that a number of genes residing on chromosome 11 might be involved in the regulation of muscle fiber phenotypes.

The QTL affecting the CSA of type I and type IIA fibers on chromosome 6, albeit at 1% chromosome-wide threshold of significance (**Table 1**), overlapped with the *Mfq2* locus found in the  $F_2$  population, characterized by the same increasing allele, LG/J. Thus, the support interval of *Mfq2* could be considered to be 5.18 Mb rather than the previously reported 56.5 Mb. Importantly, the immediate proximity of the refined region (Chr 6: 110.8-116.0 Mb) to the syntenic region (Chr 6:116.0-118.0 Mb) implicated in the QTL affecting the diameter of pig IIA fibers (17) suggest that the same genes could be underlying the effects of these QTLs in mice.

A QTL affecting percentage of type I fibers (at 10% chromosome-wide threshold) on chromosome 1 (67.6to 70.8 Mb) overlapped with *Mfq1* locus which influenced the CSA of type I and type IIA fiber area in the  $F_2$  population (11). However, because the CSA and percentage of type I fibers are poorly correlated traits both in the  $F_{34}$  (**Supplementary Table**

4) and the F<sub>2</sub> mice (11), it is likely that different genes are underlying the *Mfq1* locus and the QTL identified in the F<sub>34</sub> population. Further studies are required to clarify this observation.

### **Transcriptome analysis**

In the present study, the expressed transcriptome in soleus muscle of the parental strains was examined in order to facilitate nomination of the candidate genes within the refined QTL. We hypothesized that if the phenotypic effect of the QTL was brought about by the allele specific abundance of transcripts encoded by genes within the QTL, such genes would be differentially expressed in the transcriptome between the parental strains. Comparison of expression of the genes within the four most robust QTLs identified *Alad* and *Hdhd3* genes as potential candidates for the *Mfq6* locus, which affects the proportion of type I fibers. Transcripts of both genes are more abundant in the LG/J compared to the SM/J strain. This is consistent with our findings in the TA muscle of the same strains (48). Of these two identified candidate genes, transcripts of *Alad* are ~20 times more abundant in the mouse muscle than *Hdhd3*, regardless of strain. In addition, *Alad* may play a role during myogenesis as its expression in C2C12 myogenic cells is 5-fold higher compared to differentiated muscle (79).

### **Candidate genes.**

The support intervals of four most robust QTLs harbor 159 genes (**Supplementary Table 2**). These regions were scrutinized further for the genes fulfilling one of the following criteria: presence of the functional variants (i.e. non-synonymous SNPs predicted to alter function of encoded protein); abundance of transcript in muscle lineage, particularly in comparison to other tissues and cell types; differential expression in the soleus of the two strains; and by comparing genomic sequence between the LG/J and SM/J strains a list of 21 genes was highlighted (**Supplementary Table 3**) with the strain-specific functional variants. Using bioinformatics, 4 genes abundantly and/or preferentially expressed in skeletal muscle compared to other types of tissues and cells were identified. Our own analysis of gene

expression in soleus muscle highlighted a set of 20 genes differentially expressed between the two strains (**Table 2**). Intersection of all these lists permitted us to prioritise nine candidate genes which appeared on more than one of these lists and/or for which independent and accessible validation models were available (i.e. *Chd6* and *Alad*). Because neither the *Chd6* (**Figure 4**) nor *Alad* genes were found to affect proportion of type I fibres in the way predicted by the QTL analyses, the list of prioritised candidates was reduced to 7 genes annotated in **Supplementary Table 5**. Three out of four QTLs contain one (*Mfq6*) or more candidate genes. All candidates are abundantly transcribed in muscle lineage with *Psme4*, *Acyp2* and *Mafb* showing the highest level of expression in skeletal muscle compared to other tissues and cells. None of the seven candidates have been previously implicated to affect properties of skeletal muscle fibres although some of them have been implicated in cardiomyopathy or function as transcription factors (**Supplementary Table 5**). Thus, genomic and gene expression analyses permitted focusing on a limited number of positional candidates in the future validation studies for establishing the causative genes.

## **Conclusion**

In conclusion, we have refined the genetic architecture affecting cross sectional area of soleus muscle fibers and proportion of type I fibers in the LG/J and SM/J derived lineage. Integrating QTL mapping, genomic and transcriptome data from homologous muscle highlighted several candidate genes that may underpin muscle phenotypes critical to health and disease and worthy of follow up analyses.

Grants

442 The study was supported by NIH R01AR056280 grant (AL), NIH R01DA024845 (AAP) and  
443 Marie Curie International Reintegration Grant 249156 (AL).

444

#### 445 Acknowledgements

446 The authors wish to acknowledge technical assistance from Diane Stewart, Jasmine C.  
447 Berry and Jake T. Brown. We acknowledge the Dartmouth Mouse Modelling Shared  
448 Resource of the Norris Cotton Cancer Center (support grant P30CA023108-27), for  
449 providing *Chd6* knockout mouse samples.

450

#### 451 Author contributions

452 A.L. conceived and supervised the study, A.M.C. phenotyped muscle samples, A.A.P.  
453 provided genotypes and oversaw the QTL analyses, R.C. designed the QTLRel software  
454 used in the QTL analysis, A.M.C. carried out QTL mapping, M.E.S, C.M. and E.C.D. did  
455 transcriptome analysis, S.N.F. and J.L.F. generated and provided *Chd6* knockout samples,  
456 A.L. and A.M.C. wrote the manuscript with input from all co-authors.

457

458

## Figure legends

**Figure 1. Individual variability in proportion of oxidative fibers.** Representative images of F<sub>34</sub> female soleus cross-sections following myosin ATPase staining (acid pre-incubation). Dark fibers type I, pale fibers type IIA.

**Figure 2. Type I fiber cross-sectional area QTL on chromosome 11.** Analyses were carried out in the F<sub>2</sub> intercross and in the combined F<sub>2</sub> and F<sub>34</sub> populations. X-axis indicates the relative position in the linkage map in centimorgan (cM). The thresholds are at the level of 0.05 genome wise significance for the F<sub>2</sub> output (dotted line) and combined output (solid line).

**Figure 3. Sex specificity of *Mfq3* locus on cross-sectional area (CSA) of soleus type I and IIA fibers in the F<sub>34</sub> intercross.** Mean and SEM. Genotype at the peak marker: LG, homozygous for LG/J allele; H, heterozygous; SM, homozygous for SM/J allele.

**Figure4. Percentage of type I fibers in the soleus muscle of 4 month old *Chd6* knockout (KO), heterozygous (HET) and wild-type (WT) females.** There is no difference in percentage of type I muscle fibers in the soleus muscle between knockout, heterozygotes and wild-type groups (P=0.3041). Each data point is from a single mouse, horizontal lines represent group mean.

481 **Table 1.** Characteristics of muscle fiber QTL in combined analyses of the F<sub>2</sub> and F<sub>34</sub>  
 482 intercrosses derived from the LG/J and SM/J strains.

Chr	Thr**	Level***	Start Mb <sup>†</sup>	End Mb	Size Mb	Trait	Locus¥
1	C	0.1	67.6	70.7	3.1	% Type I	
1	C	0.1	193.9	194.3	0.4	% Type I & CSA2A	
2	C	0.1	92.4	104.8	12.4	% Type I	
2	C	0.05	139.6	145.6	6.0	% Type I	
<b>2</b>	<b>G</b>	<b>0.01</b>	158.8	162.5	3.7	<b>% Type I</b>	<b>Mfq4 (SM)</b>
<b>3</b>	<b>G</b>	<b>0.05</b>	<b>33.6</b>	<b>40.0</b>	<b>6.4</b>	<b>CSA1 &amp; CSA2A</b>	<b>Mfq5 (SM)</b>
<b>4</b>	<b>G</b>	<b>0.05</b>	<b>57.7</b>	<b>62.7</b>	<b>5.0</b>	<b>% Type I</b>	<b>Mfq6 (LG)</b>
4	C	0.05	103.9	106.1	2.2	% Type I	
6	C	0.05	81.9	84.1	2.2	CSAIIA	
6	C	0.01	110.8	116.0	5.2	CSA1 & CSA2A	Mfq2* (LG)
7	C	0.05	138.4	140.0	1.6	% Type I	
8	C	0.1	7.4	12.4	5.0	% Type I	
8	C	0.05	89.0	92.4	3.4	TOTAL	
8	C	0.01	121.9	128.6	6.7	TOTAL	
10	G	0.1	120.7	121.3	0.6	% Type I	
11	C	0.1	12.4	17.2	4.8	CSAIIA	
11	C	0.1	19.1	23.1	4.0	CSAIIA	
<b>11</b>	<b>G</b>	<b>0.01</b>	<b>28.0</b>	<b>31.5</b>	<b>3.5</b>	<b>CSA1 &amp; CSA2A</b>	<b>Mfq3* (LG)</b>
11	C	0.1	62.5	64.2	1.7	% Type I	
11	C	0.1	70.6	76.2	5.6	% Type I	
12	C	0.1	27.6	29.3	1.7	CSA1	
13	C	0.01	5.3	9.9	4.6	% Type I	
13	C	0.05	71.5	74.0	2.5	CSAIIA	
14	C	0.05	93.6	102.3	8.7	CSAIIA	
15	C	0.1	12.1	20.3	8.2	TOTAL	
16	C	0.05	68.9	75.1	6.2	CSA1 & CSA2A	
X	C	0.01	11.8	52.5	40.7	TOTAL	

483 \* refined previously identified QTL in the LG/J and SM/J F<sub>2</sub> intercross (47).

484 \*\* C – chromosome-wide threshold, G- genome-wide threshold

485 \*\*\* Level of significance

486 ¥ LG –increasing allele is LG/J, SM- increasing allele is SM/J

487 <sup>†</sup> Genomic positions based on GRCm38.p3.

488

489 **Table 2.** Positional candidate genes differentially expressed between LG/J and SM/J soleus muscles.

Chr	QTL	Probe set ID	Gene	p-value*	Fold-Change**	Gene name***
2	<i>Mfq4</i>	17393868	<b><i>Mafb</i></b>	0.033	-1.77	v-maf musculoaponeurotic fibrosarcoma oncogene family, protein B (avian)
		17393910	<i>Chd6</i>	0.042724	-1.13	chromodomain helicase DNA binding protein 6
		17404652	<i>Gm24780</i>	0.032914	-1.98	Predicted gene Gm24780, predicted protein is B4HDV3.
3	<i>Mfq5</i>	17396801	<i>Ttc14</i>	0.033387	-1.14	tetratricopeptide repeat domain 14
		17396876	----	0.0219376	-1.80	There are no assigned mRNA sequences for this probe set. The probe set lies within lncRNA Sox2ot (Sox2 overlapping transcript, non-protein coding)
		17425606	<i>Gm12526</i>	0.046791	-1.17	predicted gene 12526
		17414380	<i>Gm24277</i>	0.00525644	-2.07	Gm24277 a known snRNA. The probeset also lies within an intronic region of RefSeq gene Pakap (PALM2-AKAP2), a read through transcript on chromosome 4
		17425701	<i>Mir3095</i>	0.0298235	-1.78	Mir3095 (Entrez ID 100526502; EST ENSMUST00000175552).
		17426097	<i>Mup3</i>	0.00304	-1.49	major urinary protein 3
		17426126	<i>Fkbp15</i>	0.018038	-1.10	FK506 binding protein 15
4	<i>Mfq6</i>	17414545	<i>Slc31a1</i>	0.049799	1.12	solute carrier family 31, member 1
		17426166	<i>Cdc26</i>	0.021407	-1.29	cell division cycle 26
		17426198	<i>Hdhd3</i>	0.000869	1.86	haloacid dehalogenase-like hydrolase domain containing 3
		17426206	<i>Alad</i>	9.75E-05	1.91	aminolevulinate, delta-, dehydratase
		17414600	<i>Rgs3</i>	0.023096	1.08	regulator of G-protein signaling 3
		17248064	<b><i>Mtif2</i></b>	0.015517	-1.16	mitochondrial translational initiation factor 2
		17261285	<i>LOC102637613</i>	0.00432682	1.68	linc RNA [AK084560 (EST)/ Gm12092 (predicted gene)].
		17248127	----	0.00187422	-1.46	There are no assigned mRNA sequences for this transcript. The probe set lies within an intron of <i>Sptbn1</i> .
		17261393	<b><i>Acyp2</i></b>	0.011777	1.26	acylphosphatase 2, muscle type
		17248196	<i>Asb3</i>	0.014221	-1.15	ankyrin repeat and SOCS box-containing 3

491 \* ANOVA p-value for strain effect; \*\* Fold change uses SM/J as baseline (negative values indicate LG/J expression is down compared to SM/J,  
492 positive values LG/J expression up compared to SM/J); bold indicates that gene is predominantly and/ or strongly expressed in skeletal muscle  
493 tissue (79). \*\*\* For probe sets not designed against an annotated gene, genes at the genomic loci of the Affymetrix probeset were identified in  
494 UCSC genome browser using mouse genome build GRCm38.

495



497

- 498 1. **Abe T, Saburi J, Hasebe H, Nakagawa T, Kawamura T, Saito K, Nade T, Misumi S, Okumura**  
499 **T, Kuchida K, Hayashi T, Nakane S, Mitsuhashi T, Nirasawa K, Sugimoto Y, and Kobayashi E.** Bovine  
500 quantitative trait loci analysis for growth, carcass, and meat quality traits in an F2 population from a  
501 cross between Japanese Black and Limousin. *Journal of animal science* 86: 2821-2832, 2008.
- 502 2. **Allen DL, Sartorius CA, Sycuro LK, and Leinwand LA.** Different pathways regulate expression  
503 of the skeletal myosin heavy chain genes. *The Journal of biological chemistry* 276: 43524-43533,  
504 2001.
- 505 3. **Bishop TR, Miller MW, Wang A, and Dierks PM.** Multiple copies of the ALA-D gene are  
506 located at the Lv locus in Mus domesticus mice. *Genomics* 48: 221-231, 1998.
- 507 4. **Blanco G, Coulton GR, Biggin A, Grainge C, Moss J, Barrett M, Berquin A, Marechal G,**  
508 **Skyner M, van Mier P, Nikitopoulou A, Kraus M, Ponting CP, Mason RM, and Brown SD.** The  
509 kyphoscoliosis (ky) mouse is deficient in hypertrophic responses and is caused by a mutation in a  
510 novel muscle-specific protein. *Human molecular genetics* 10: 9-16, 2001.
- 511 5. **Bordet T, Schmalbruch H, Pettmann B, Hagege A, Castelnau-Ptakhine L, Kahn A, and Haase**  
512 **G.** Adenoviral cardiotoxin-1 gene transfer protects pmn mice from progressive motor  
513 neuronopathy. *The Journal of clinical investigation* 104: 1077-1085, 1999.
- 514 6. **Bottinelli R, and Reggiani C.** Human skeletal muscle fibres: molecular and functional  
515 diversity. *Progress in biophysics and molecular biology* 73: 195-262, 2000.
- 516 7. **Briguet A, Courdier-Fruh I, Foster M, Meier T, and Magyar JP.** Histological parameters for  
517 the quantitative assessment of muscular dystrophy in the mdx-mouse. *Neuromuscular disorders* :  
518 *NMD* 14: 675-682, 2004.
- 519 8. **Brooke MH, and Kaiser KK.** Muscle fiber types: how many and what kind? *Archives of*  
520 *neurology* 23: 369-379, 1970.
- 521 9. **Carbonetto P, Cheng R, Gyekis JP, Parker CC, Blizard DA, Palmer AA, and Lionikas A.**  
522 Discovery and refinement of muscle weight QTLs in B6 × D2 advanced intercross mice. *Physiological*  
523 *Genomics* 46: 571-582, 2014.
- 524 10. **Carpenter CE, Rice OD, Cockett NE, and Snowden GD.** Histology and composition of muscles  
525 from normal and callipyge lambs. *Journal of animal science* 74: 388-393, 1996.
- 526 11. **Carroll AM, Palmer AA, and Lionikas A.** QTL Analysis of Type I and Type IIA Fibers in Soleus  
527 Muscle in a Cross between LG/J and SM/J Mouse Strains. *Frontiers in genetics* 2: 99, 2011.
- 528 12. **Cheng R, Abney M, Palmer AA, and Skol AD.** QTLRel: an R package for genome-wide  
529 association studies in which relatedness is a concern. *BMC genetics* 12: 66, 2011.
- 530 13. **Cheng R, Lim JE, Samocha KE, Sokoloff G, Abney M, Skol AD, and Palmer AA.** Genome-wide  
531 association studies and the problem of relatedness among advanced intercross lines and other  
532 highly recombinant populations. *Genetics* 185: 1033-1044, 2010.
- 533 14. **Cheng R, and Palmer AA.** A Simulation Study of Permutation, Bootstrap, and Gene Dropping  
534 for Assessing Statistical Significance in the Case of Unequal Relatedness. *Genetics* 193: 1015-1018,  
535 2013.
- 536 15. **Costill DL, Daniels J, Evans W, Fink W, Krahenbuhl G, and Saltin B.** Skeletal muscle enzymes  
537 and fiber composition in male and female track athletes. *Journal of applied physiology* 40: 149-154,  
538 1976.
- 539 16. **Darvasi A, and Soller M.** Advanced intercross lines, an experimental population for fine  
540 genetic mapping. *Genetics* 141: 1199-1207, 1995.
- 541 17. **Estelle J, Gil F, Vazquez JM, Latorre R, Ramirez G, Barragan MC, Folch JM, Noguera JL, Toro**  
542 **MA, and Perez-Enciso M.** A quantitative trait locus genome scan for porcine muscle fiber traits  
543 reveals overdominance and epistasis. *Journal of animal science* 86: 3290-3299, 2008.

- 544 18. **Fink WJ, Costill DL, and Pollock ML.** Submaximal and maximal working capacity of elite  
545 distance runners. Part II. Muscle fiber composition and enzyme activities. *Annals of the New York*  
546 *Academy of Sciences* 301: 323-327, 1977.
- 547 19. **Frederiksen H, Gaist D, Petersen HC, Hjelmberg J, McGue M, Vaupel JW, and Christensen K.**  
548 Hand grip strength: a phenotype suitable for identifying genetic variants affecting mid- and late-life  
549 physical functioning. *Genetic epidemiology* 23: 110-122, 2002.
- 550 20. **Girgenrath S, Song K, and Whittemore LA.** Loss of myostatin expression alters fiber-type  
551 distribution and expression of myosin heavy chain isoforms in slow- and fast-type skeletal muscle.  
552 *Muscle & nerve* 31: 34-40, 2005.
- 553 21. **Guderley H, Houle-Leroy P, Diffie GM, Camp DM, and Garland T, Jr.** Morphometry,  
554 ultrastructure, myosin isoforms, and metabolic capacities of the "mini muscles" favoured by  
555 selection for high activity in house mice. *Comparative biochemistry and physiology Part B,*  
556 *Biochemistry & molecular biology* 144: 271-282, 2006.
- 557 22. **Guderley H, Joanisse DR, Mokas S, Bilodeau GM, and Garland T, Jr.** Altered fibre types in  
558 gastrocnemius muscle of high wheel-running selected mice with mini-muscle phenotypes.  
559 *Comparative biochemistry and physiology Part B, Biochemistry & molecular biology* 149: 490-500,  
560 2008.
- 561 23. **Hartmann J, Garland T, Jr., Hannon RM, Kelly SA, Munoz G, and Pomp D.** Fine mapping of  
562 "mini-muscle," a recessive mutation causing reduced hindlimb muscle mass in mice. *The Journal of*  
563 *heredity* 99: 679-687, 2008.
- 564 24. **Hernelahti M, Tikkanen HO, Karjalainen J, and Kujala UM.** Muscle fiber-type distribution as  
565 a predictor of blood pressure: a 19-year follow-up study. *Hypertension* 45: 1019-1023, 2005.
- 566 25. **Hubal MJ, Gordish-Dressman H, Thompson PD, Price TB, Hoffman EP, Angelopoulos TJ,**  
567 **Gordon PM, Moyna NM, Pescatello LS, Visich PS, Zoeller RF, Seip RL, and Clarkson PM.** Variability in  
568 muscle size and strength gain after unilateral resistance training. *Medicine and science in sports and*  
569 *exercise* 37: 964-972, 2005.
- 570 26. **Huygens W, Thomis MA, Peeters MW, Vlietinck RF, and Beunen GP.** Determinants and  
571 upper-limit heritabilities of skeletal muscle mass and strength. *Canadian journal of applied*  
572 *physiology = Revue canadienne de physiologie appliquee* 29: 186-200, 2004.
- 573 27. **Jaworowski A, Porter MM, Holmback AM, Downham D, and Lexell J.** Enzyme activities in  
574 the tibialis anterior muscle of young moderately active men and women: relationship with body  
575 composition, muscle cross-sectional area and fibre type composition. *Acta physiologica Scandinavica*  
576 176: 215-225, 2002.
- 577 28. **Kadi F, Eriksson A, Holmner S, Butler-Browne GS, and Thornell LE.** Cellular adaptation of the  
578 trapezius muscle in strength-trained athletes. *Histochemistry and cell biology* 111: 189-195, 1999.
- 579 29. **Karjalainen J, Tikkanen H, Hernelahti M, and Kujala UM.** Muscle fiber-type distribution  
580 predicts weight gain and unfavorable left ventricular geometry: a 19 year follow-up study. *BMC*  
581 *cardiovascular disorders* 6: 2, 2006.
- 582 30. **Karp CL, Grupe A, Schadt E, Ewart SL, Keane-Moore M, Cuomo PJ, Kohl J, Wahl L,**  
583 **Kuperman D, Germer S, Aud D, Peltz G, and Wills-Karp M.** Identification of complement factor 5 as  
584 a susceptibility locus for experimental allergic asthma. *Nature immunology* 1: 221-226, 2000.
- 585 31. **Kelly SA, Bell TA, Selitsky SR, Buus RJ, Hua K, Weinstock GM, Garland T, Pardo-Manuel de**  
586 **Villena F, and Pomp D.** A Novel Intronic Single Nucleotide Polymorphism in the Myosin heavy  
587 polypeptide 4 Gene Is Responsible for the Mini-Muscle Phenotype Characterized by Major Reduction  
588 in Hind-Limb Muscle Mass in Mice. *Genetics* 195: 1385-1395, 2013.
- 589 32. **Kilikevicius A, Venckunas T, Zelniene R, Carroll AM, Lionikaite S, Ratkevicius A, and**  
590 **Lionikas A.** Divergent physiological characteristics and responses to endurance training among  
591 inbred mouse strains. *Scandinavian journal of medicine & science in sports* 23: 657-668, 2013.
- 592 33. **Kim J, Wang Z, Heymsfield SB, Baumgartner RN, and Gallagher D.** Total-body skeletal  
593 muscle mass: estimation by a new dual-energy X-ray absorptiometry method. *The American journal*  
594 *of clinical nutrition* 76: 378-383, 2002.

595 34. **Kitamura T, Kitamura YI, Funahashi Y, Shawber CJ, Castrillon DH, Kollipara R, DePinho RA,**  
596 **Kitajewski J, and Accili D.** A Foxo/Notch pathway controls myogenic differentiation and fiber type  
597 specification. *The Journal of clinical investigation* 117: 2477-2485, 2007.

598 35. **Klein RF, Allard J, Avnur Z, Nikolcheva T, Rotstein D, Carlos AS, Shea M, Waters RV,**  
599 **Belknap JK, Peltz G, and Orwoll ES.** Regulation of bone mass in mice by the lipoxigenase gene  
600 Alox15. *Science (New York, NY)* 303: 229-232, 2004.

601 36. **Klover P, Chen W, Zhu BM, and Hennighausen L.** Skeletal muscle growth and fiber  
602 composition in mice are regulated through the transcription factors STAT5a/b: linking growth  
603 hormone to the androgen receptor. *FASEB journal : official publication of the Federation of American*  
604 *Societies for Experimental Biology* 23: 3140-3148, 2009.

605 37. **Komi PV, Viitasalo JH, Havu M, Thorstensson A, Sjodin B, and Karlsson J.** Skeletal muscle  
606 fibres and muscle enzyme activities in monozygous and dizygous twins of both sexes. *Acta*  
607 *physiologica Scandinavica* 100: 385-392, 1977.

608 38. **Koohmaraie M, Shackelford SD, Wheeler TL, Lonergan SM, and Doumit ME.** A muscle  
609 hypertrophy condition in lamb (callipyge): characterization of effects on muscle growth and meat  
610 quality traits. *Journal of animal science* 73: 3596-3607, 1995.

611 39. **Lander E, and Kruglyak L.** Genetic dissection of complex traits: guidelines for interpreting  
612 and reporting linkage results. *Nature genetics* 11: 241-247, 1995.

613 40. **Lathrop MJ, Chakrabarti L, Eng J, Harker Rhodes C, Lutz T, Nieto A, Denny Liggitt H, Warner**  
614 **S, Fields J, Stöger R, and Fiering S.** Deletion of the Chd6 exon 12 affects motor coordination.  
615 *Mammalian Genome* 21: 130-142, 2010.

616 41. **Lee KY, Singh MK, Ussar S, Wetzel P, Hirshman MF, Goodyear LJ, Kispert A, and Kahn CR.**  
617 Tbx15 controls skeletal muscle fibre-type determination and muscle metabolism. *Nature*  
618 *communications* 6: 8054, 2015.

619 42. **Leick L, Hellsten Y, Fentz J, Lyngby SS, Wojtaszewski JF, Hidalgo J, and Pilegaard H.** PGC-  
620 1alpha mediates exercise-induced skeletal muscle VEGF expression in mice. *American journal of*  
621 *physiology Endocrinology and metabolism* 297: E92-103, 2009.

622 43. **Li WB, Ren J, Zhu WC, Guo BL, Yang B, Liu LT, Ding NS, Ma JW, Li L, and Huang LS.** Mapping  
623 QTL for porcine muscle fibre traits in a White Duroc x Erhualian F(2) resource population. *Journal of*  
624 *animal breeding and genetics = Zeitschrift fur Tierzucht und Zuchtungsbiologie* 126: 468-474,  
625 2009.

626 44. **Lillioja S, Young AA, Culter CL, Ivy JL, Abbott WG, Zawadzki JK, Yki-Jarvinen H, Christin L,**  
627 **Secomb TW, and Bogardus C.** Skeletal muscle capillary density and fiber type are possible  
628 determinants of in vivo insulin resistance in man. *The Journal of clinical investigation* 80: 415-424,  
629 1987.

630 45. **Lionikas A, Blizard DA, Gerhard GS, Vandenberg DJ, Stout JT, Vogler GP, McClearn GE, and**  
631 **Larsson L.** Genetic determinants of weight of fast- and slow-twitch skeletal muscle in 500-day-old  
632 mice of the C57BL/6J and DBA/2J lineage. *Physiological genomics* 21: 184-192, 2005.

633 46. **Lionikas A, Blizard DA, Vandenberg DJ, Glover MG, Stout JT, Vogler GP, McClearn GE, and**  
634 **Larsson L.** Genetic architecture of fast- and slow-twitch skeletal muscle weight in 200-day-old mice  
635 of the C57BL/6J and DBA/2J lineage. *Physiological genomics* 16: 141-152, 2003.

636 47. **Lionikas A, Cheng R, Lim JE, Palmer AA, and Blizard DA.** Fine-mapping of muscle weight QTL  
637 in LG/J and SM/J intercrosses. *Physiological genomics* 42a: 33-38, 2010.

638 48. **Lionikas A, Meharg C, Derry JM, Ratkevicius A, Carroll AM, Vandenberg DJ, and Blizard**  
639 **DA.** Resolving candidate genes of mouse skeletal muscle QTL via RNA-Seq and expression network  
640 analyses. *BMC genomics* 13: 592, 2012.

641 49. **Luff AR, and Goldspink G.** Total number of fibers in muscles of several strains of mice.  
642 *Journal of animal science* 30: 891-893, 1970.

643 50. **MacArthur DG, Seto JT, Chan S, Quinlan KG, Raftery JM, Turner N, Nicholson MD, Kee AJ,**  
644 **Hardeman EC, Gunning PW, Cooney GJ, Head SI, Yang N, and North KN.** An Actn3 knockout mouse

provides mechanistic insights into the association between alpha-actinin-3 deficiency and human athletic performance. *Human molecular genetics* 17: 1076-1086, 2008.

51. **MacDougall JD, Sale DG, Alway SE, and Sutton JR.** Muscle fiber number in biceps brachii in bodybuilders and control subjects. *Journal of applied physiology: respiratory, environmental and exercise physiology* 57: 1399-1403, 1984.

52. **Malek M, Dekkers JC, Lee HK, Baas TJ, Prusa K, Huff-Lonergan E, and Rothschild MF.** A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig. II. Meat and muscle composition. *Mammalian genome : official journal of the International Mammalian Genome Society* 12: 637-645, 2001.

53. **Mannion AF, Dumas GA, Cooper RG, Espinosa FJ, Faris MW, and Stevenson JM.** Muscle fibre size and type distribution in thoracic and lumbar regions of erector spinae in healthy subjects without low back pain: normal values and sex differences. *Journal of anatomy* 190 ( Pt 4): 505-513, 1997.

54. **McPherron AC, Lawler AM, and Lee SJ.** Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. *Nature* 387: 83-90, 1997.

55. **Nii M, Hayashi T, Mikawa S, Tani F, Niki A, Mori N, Uchida Y, Fujishima-Kanaya N, Komatsu M, and Awata T.** Quantitative trait loci mapping for meat quality and muscle fiber traits in a Japanese wild boar x Large White intercross. *Journal of animal science* 83: 308-315, 2005.

56. **Nikolskiy I, Conrad DF, Chun S, Fay JC, Cheverud JM, and Lawson HA.** Using whole-genome sequences of the LG/J and SM/J inbred mouse strains to prioritize quantitative trait genes and nucleotides. *BMC genomics* 16: 415, 2015.

57. **Nimmo MA, Wilson RH, and Snow DH.** The inheritance of skeletal muscle fibre composition in mice. *Comparative biochemistry and physiology A, Comparative physiology* 81: 109-115, 1985.

58. **Pourhassan M, Bosy-Westphal A, Schautz B, Braun W, Gluer CC, and Muller MJ.** Impact of body composition during weight change on resting energy expenditure and homeostasis model assessment index in overweight nonsmoking adults. *The American journal of clinical nutrition* 99: 779-791, 2014.

59. **Rehfeldt C, Ott G, Gerrard DE, Varga L, Schlote W, Williams JL, Renne U, and Bunger L.** Effects of the compact mutant myostatin allele Mstn (Cmpt-dl1Abc) introgressed into a high growth mouse line on skeletal muscle cellularity. *Journal of muscle research and cell motility* 26: 103-112, 2005.

60. **Roepstorff C, Donsmark M, Thiele M, Vistisen B, Stewart G, Vissing K, Schjerling P, Hardie DG, Galbo H, and Kiens B.** Sex differences in hormone-sensitive lipase expression, activity, and phosphorylation in skeletal muscle at rest and during exercise. *American journal of physiology Endocrinology and metabolism* 291: E1106-1114, 2006.

61. **Sainz N, Rodriguez A, Catalan V, Becerril S, Ramirez B, Gomez-Ambrosi J, and Fruhbeck G.** Leptin administration favors muscle mass accretion by decreasing FoxO3a and increasing PGC-1alpha in ob/ob mice. *PloS one* 4: e6808, 2009.

62. **Schiaffino S.** Fibre types in skeletal muscle: a personal account. *Acta physiologica (Oxford, England)* 199: 451-463, 2010.

63. **Simoneau JA, and Bouchard C.** Genetic determinism of fiber type proportion in human skeletal muscle. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 9: 1091-1095, 1995.

64. **Simoneau JA, and Bouchard C.** Human variation in skeletal muscle fiber-type proportion and enzyme activities. *The American journal of physiology* 257: E567-572, 1989.

65. **Sosnicki A.** Histopathological observation of stress myopathy in M. longissimus in the pig and relationships with meat quality, fattening and slaughter traits. *Journal of animal science* 65: 584-596, 1987.

66. **Srikanthan P, and Karlamangla AS.** Muscle Mass Index As a Predictor of Longevity in Older Adults. *The American Journal of Medicine* 127: 547-553, 2014.

67. **Staron RS, Hagerman FC, Hikida RS, Murray TF, Hostler DP, Crill MT, Ragg KE, and Toma K.** Fiber type composition of the vastus lateralis muscle of young men and women. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society* 48: 623-629, 2000.
68. **Stavaux D, Art T, McEntee K, Reznick M, and Lekeux P.** Muscle fibre type and size, and muscle capillary density in young double-muscled blue Belgian cattle. *Zentralblatt für Veterinärmedizin Reihe A* 41: 229-236, 1994.
69. **Timson BF, Bowlin BK, Dudenhoeffer GA, and George JB.** Fiber number, area, and composition of mouse soleus muscle following enlargement. *Journal of applied physiology (Bethesda, Md : 1985)* 58: 619-624, 1985.
70. **Totsuka Y, Nagao Y, Horii T, Yonekawa H, Imai H, Hatta H, Izaike Y, Tokunaga T, and Atomi Y.** Physical performance and soleus muscle fiber composition in wild-derived and laboratory inbred mouse strains. *Journal of applied physiology (Bethesda, Md : 1985)* 95: 720-727, 2003.
71. **Van den Maagdenberg K, Stinckens A, Lefaucheur L, Buys N, and De Smet S.** The effect of mutations in the insulin-like growth factor-II and ryanodine receptor-1 genes on biochemical and histochemical muscle fibre characteristics in pigs. *Meat science* 79: 757-766, 2008.
72. **van der Laarse WJ, Diegenbach PC, and Maslam S.** Quantitative histochemistry of three mouse hind-limb muscles: the relationship between calcium-stimulated myofibrillar ATPase and succinate dehydrogenase activities. *The Histochemical journal* 16: 529-541, 1984.
73. **Vaughan HS, Aziz U, Goldspink G, and Nowell NW.** Sex and stock differences in the histochemical myofibrillar adenosine triphosphatase reaction in the soleus muscle of the mouse. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society* 22: 155-159, 1974.
74. **Wade AJ, Marbut MM, and Round JM.** Muscle fibre type and aetiology of obesity. *Lancet* 335: 805-808, 1990.
75. **Wang YX, Zhang CL, Yu RT, Cho HK, Nelson MC, Bayuga-Ocampo CR, Ham J, Kang H, and Evans RM.** Regulation of muscle fiber type and running endurance by PPARdelta. *PLoS biology* 2: e294, 2004.
76. **Wilkins BJ, Dai YS, Bueno OF, Parsons SA, Xu J, Plank DM, Jones F, Kimball TR, and Molkentin JD.** Calcineurin/NFAT coupling participates in pathological, but not physiological, cardiac hypertrophy. *Circulation research* 94: 110-118, 2004.
77. **Wimmers K, Fiedler I, Hardge T, Murani E, Schellander K, and Ponsuksili S.** QTL for microstructural and biophysical muscle properties and body composition in pigs. *BMC genetics* 7: 15, 2006.
78. **Wirtz P, Loermans HM, Peer PG, and Reintjes AG.** Postnatal growth and differentiation of muscle fibres in the mouse. I. A histochemical and morphometrical investigation of normal muscle. *Journal of anatomy* 137 (Pt 1): 109-126, 1983.
79. **Wu C, Orozco C, Boyer J, Leglise M, Goodale J, Batalov S, Hodge CL, Haase J, Janes J, Huss JW, 3rd, and Su AI.** BioGPS: an extensible and customizable portal for querying and organizing gene annotation resources. *Genome biology* 10: R130, 2009.
80. **Zhang Y, Xu P, Lu C, Kuang Y, Zhang X, Cao D, Li C, Chang Y, Hou N, Li H, Wang S, and Sun X.** Genetic linkage mapping and analysis of muscle fiber-related QTLs in common carp (*Cyprinus carpio* L.). *Marine biotechnology (New York, NY)* 13: 376-392, 2011.

**A**



**B**



**500  $\mu$ m**

## Chromosome 11







